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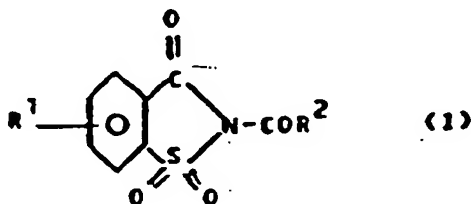
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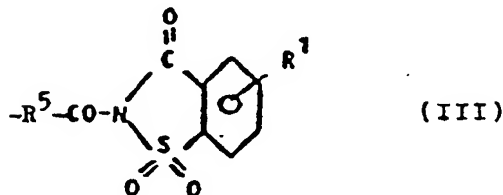
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(54) **Benzisothiazolinone-1-dioxide derivatives as elastase inhibitors.**

(57) The invention relates to pharmaceutical and cosmetic compositions comprising at least one benzisothiazolinone-1-dioxide derivative with the formula:



where R¹ can be hydrogen, and R² can be certain generally hydrophobic C₈-C₂₀ aliphatic groups or the group:



where R⁵ is aliphatic or else R² is a saturated or unsaturated C₂-C₆ group ending in an aromatic nucleus. These compositions can be used to inhibit elastases such as human leucocytic elastase. They may be applied topically as a cosmetic.

Many of the benzisothiazolinone-1-dioxide derivative specified as active ingredient in such compositions are novel compounds.

EP 0 446 047 A1

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BENZISOTHIAZOLINONE-1-DIOXIDE DERIVATIVES AS ELASTASE INHIBITORS

The present invention relates to benzisothiazolinone-1-dioxide derivatives that can be used as elastase inhibitors, and to compositions containing such inhibitors.

It is well known that elastin is an elastic fibrous protein that occurs in the connective tissues of vertebrates. It is found in the walls of the blood vessels, the skin, lungs, cartilage, ligaments and other tissues. Elastin is the most durable protein in the body, but it suffers a particularly rapid degradation in all the elastin-rich tissues. Such as the vascular walls and the skin, in certain pathological conditions, as well as during the ageing process in general.

Elastin can be attacked only by certain proteases, called elastases or elastase-type proteases. These enzymes include pancreatic elastase and cell elastases, examples of the latter being leucocytic and platelet elastases, as well as the elastases found in macrophages, fibroblasts and the cells of the smooth muscles in the arteries. These enzymes can degrade the elastin in the tissues and organs mentioned above and so contribute to the development of disorders such as arteriosclerosis, emphysema, arthritis and diabetes, as well as to the ageing of the connective tissues in the body.

The activity of elastases is controlled and regulated by natural inhibitors present in the plasma (e.g. α -1-antitrypsin and α -2-macroglobulin) and in secretions from tissues (e.g. the bronchial secretion) [see e.g. W. Homebeck et al., "Control of elastic tissue destruction by elastase inhibitors", in Deyl and Adam (eds.), *Connective Tissue Research: Chemistry, Biology and Physiology*, pp. 233-246, A.R. Liss Inc., New York, 1981].

Furthermore, numerous bacteria capable of entering the body secrete elastolytic proteases whose action greatly contributes to the pathogenic activity of these microorganisms.

It is also known that the growth of malignant tumours such as carcinomas and sarcomas, and the formation of metastases, which are often fatal to the patient, are also affected by the secretion of elastase-type proteases [see for example W. Homebeck, D. Brechemier, G. Bellon, J.J. Adnet and L. Robert, "Biological Significance of Elastase-like Enzymes in Arteriosclerosis and Human Breast Cancer", in P. Straulli, A.J. Barrett and A. Baici (eds.), *Proteinases and Tumor Invasion*, vol. 6, ORTC Monograph Series, pp. 117-141, Raven Press, New York, 1980]. These enzymes can destroy the surrounding tissues and thus enable the malignant cells to enter the blood circulation, as a result of which the tumour can invade the body.

For all these reasons, it is important to possess inhibitors that can control the activity of elastases.

However, some elastases are useful or even indispensable for the body, for example when they digest the bacteria that have been destroyed by the phagocytic action of macrophages. It is therefore important to possess elastase inhibitors that act selectively in the elastic fibres whose integrity is indispensable for the proper functioning of the body.

The fact is that the enzymatic hydrolysis of elastin by elastases can be seen as a decisive factor in numerous disorders of the elastic tissues, such as arteriosclerosis, emphysema and certain skin diseases. In the living body, this proteolysis occurs when the balance is upset between the level of proteases with an elastolytic action and the level of natural inhibitors originating in the plasma or the tissues. One method proposed for the treatment of a genetic or functional deficiency of these protease inhibitors is to introduce natural inhibitors such as α -1-antitrypsin as a replacement therapy.

However, the use of natural inhibitors has numerous disadvantages, including the cost of the treatment and the risk of adverse immunological reactions. Furthermore, the elastase inhibitors used in the experimental treatment of animals with emphysema are highly toxic.

Synthetic elastase-inhibitors have therefore been under investigation for some years now. Thus, US Patent Specification US-A 4,195,023 describes the use of 2-benzisothiazol-3-one derivatives and saccharin derivatives to inhibit elastases. The preferred compounds in that publication are derivatives substituted with a furoyl or a thenoyl group. The publication also gives some data for 2-(2-ethylbutyryl)-saccharin and 2-acryloylsaccharin. According to this document, the alkyl chain of the derivative contains at most 10 carbon atoms and is preferably branched, but the only example illustrated has a hydrocarbon chain with no more than five carbon atoms.

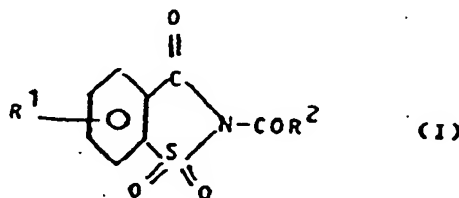
Although these inhibitors give satisfactory results, they are not sufficiently specific for the elastic fibres.

European Patent Specification EP-A 0 126,009 describes some peptide derivatives that can be used as elastase inhibitors and are specific for elastic fibres. These derivatives are lipopeptides with a hydrophobic acyl group and a special peptide chain. However, these lipopeptides, which are consequently bifunctional, have the major disadvantage of containing a peptide moiety, which is susceptible to hydrolysis by other proteinases.

The aim of the present invention is therefore to incorporate into pharmaceutical or cosmetic compositions elastase inhibitors in the form of bifunctional benzisothiazolinone-1-dioxide derivatives with a) a hydrophobic chain that has an affinity for elastin and b) a moiety that is not a peptide group (so that it is more resistant to

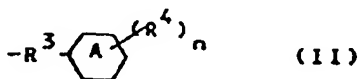
enzymatic hydrolysis) and which can also acrylate the active serine in the elastase.

The present invention therefore provides a composition comprising (i) an elastase inhibitor which is at least one benzisothiazolinone-1-dioxide derivative having the formula:



where R¹ is a hydrogen atom or a C₁-C₆ alkyl or alkoxy group, and

- 15
- R² is a monovalent C₈-C₂₀ alkyl or C₈-C₂₀ alkenyl group, optionally substituted with OH or COOH group,
 - R² is a group with the formula:



where R³ is a divalent straight or branched C₂-C₆ saturated or ethylenically unsaturated aliphatic

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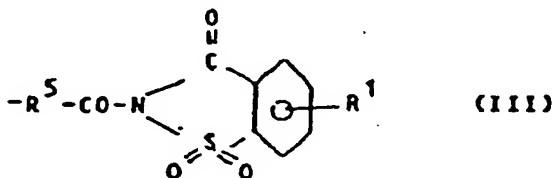
group is an aromatic nucleus

R⁴ is OH, a C₁-C₄ alkyl or a C₁-C₄ alkoxy group

n is zero or an integer in the range of 1-5, and when n > 1, the R⁴ groups can be different, or

else

- 30
- R² is a group with the formula:



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where R¹ is the same as above, and

R⁵ is a divalent straight or branched C₈-C₂₀ saturated or ethylenically unsaturated aliphatic group, and (ii) a pharmaceutically or cosmetically acceptable carrier or vehicle.

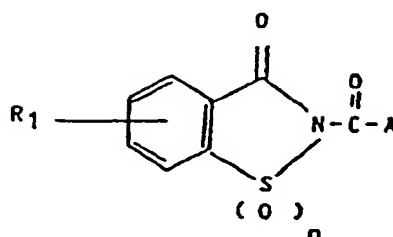
A benzisothiazolinone-1-dioxide derivative of formula (I) above, which is the active ingredient of a composition as above, has two functional groups, namely a "lipid arm" (R² or part of R²), which has a high affinity for the elastic fibres in question, and the benzisothiazolinone-1-dioxide moiety, which can react with elastase in order to inhibit it.

Owing to the presence of this hydrophobic chain, the benzisothiazolinone-1-dioxide derivatives of the present invention can accumulate on the elastic fibres to be protected and therefore act exclusively or almost exclusively on the elastase located near these fibres. In view of this mode of action, it is possible to make the substance act specifically at the site of the target fibres to be protected, which ensures a more efficient inhibition of elastase.

The compounds according to the invention are therefore more efficient and more economical bifunctional inhibitors than the known synthetic inhibitors, which lack the functional group that has a special affinity for the elastic fibres present in the tissues to be protected.

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The acylsaccharins described in US Patent Specification US-A 4,195,023, which have the formula:




carry a hydrocarbon chain A with generally at most five carbon atoms, and this is not long enough to confer on the derivative either an affinity for elastin or an ability to occupy the hydrophobic site on elastase. As will be seen later, the affinity of the derivative for elastin and for the hydrophobic site on the elastase does not become detectable until the number of carbon atoms reaches nine, improving as the number reaches eleven and peaking when this number is 16.

As mentioned in the definition of the compounds according to the invention given above, a monovalent C_8 - C_{20} straight-chain or branched alkyl group can carry at least one substituent in the form of an OH or COOH group. If the monovalent alkyl is not substituted with these groups, it preferably contains 11 carbon atoms. Such examples of such alkyl groups include the nonyl, undecyl, tridecyl, pentadecyl, heptadecyl and nonadecyl groups, while $COOH-(CH_2)_8-$ is an example of the substituted alkyl groups.

A monovalent C_9 - C_{20} alkenyl group is a monovalent alkylene group theoretically obtainable by removing a hydrogen atom from a carbon in an olefinic hydrocarbon. Such a group may contain a single ethylenic double bond or more than one ethylenic double bond. As before, these groups may be either unsubstituted or they may carry at least one OH or COOH group. Examples of such unsaturated groups are the dec-9-enyl, heptadec-8-enyl, heptadeca-8,11-dienyl and the $CH_3-(CH_2)_5-CHOH-CH_2-CH=CH-(CH_2)_7-$ group.

A divalent group R^3 may in particular be a group theoretically obtainable by removing hydrogen from each of two terminal carbons in a straight-chain or branched alkane or alkene, examples being the $-CH=CH-$ group, the $-CH_2-CH_2-$ group and the $-(CH_2)_5-$ group.

An aromatic nucleus, denoted , may comprise one or more benzene rings, examples being the groups obtained from benzene, anthracene, naphthalene, biphenyl, terphenyl, triphenylbenzene, indene, diphenylene, fluorene and phenanthrene.

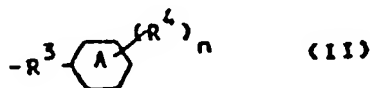
A divalent group R^5 may in particular be a group theoretically obtainable by removing a hydrogen atom from each of two terminal carbons in a straight-chain or branched C_8 - C_{20} alkane, alkene or polyalkene, as exemplified by the $-(CH_2)_8-$ group.

Furthermore, R^1 can be a hydrogen atom or a substituent chosen from amongst C_1 - C_5 alkyl or alkoxy groups. When R^1 is a substituent, it is desirably chosen such as to promote the hydrolytic opening of the heterocyclic ring. However, R^1 is generally a hydrogen atom.

As mentioned before, R^2 is the functional group that confers an affinity for the elastic fibres on the compound of the invention, owing to its lipophilic nature.

In the first embodiment of the invention, R^2 is a long-chain alkyl or alkenyl group whose long hydrocarbon chain confers a strongly lipophilic nature on the compound according to the invention. This facilitates its penetration into the skin and makes it suitable for cosmetic use.

In the second embodiment of the invention, R^2 is a group with the formula:



where R^3 and R^4 are as defined before;

R^3 is preferably a divalent group obtained from an alkane
 R^4 is a C_1 - C_4 alkoxy group or a hydroxyl group, and
 n is 1 or 2.



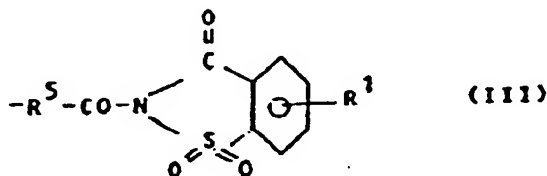
is a benzene ring

These R^2 groups are exemplified by the 3,4-dimethoxycinnamoyl, cinnamoyl, dihydrocinnamoyl and p-

methoxyphenylbutyryl radical.

In the second embodiment, the compound according to the invention is again lipophilic, this time because it comprises an aromatic nucleus.

In the third embodiment of the invention, R² has the following formula:



where R¹ and R⁵ are as defined before, but;

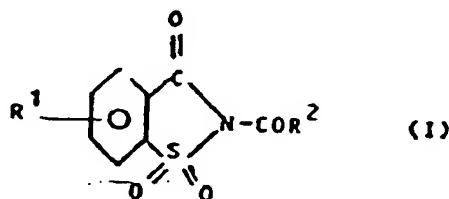
R¹ is preferably a hydrogen atom, and

R⁵ is a divalent group obtained from an alkane.

In this case, this R⁵ group confers on the compound both a lipophilic character and an affinity for the elastic fibres in question, while the presence of two benzisothiazolinone-1-dioxide rings increases the inhibitory activity.

The benzisothiazolinone-1-dioxide derivatives of this invention can be prepared by conventional methods in which the starting materials are an acid chloride and an alkali metal derivative of the corresponding benzisothiazolinone-1-dioxide.

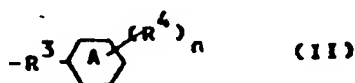
Thus, it is possible to prepare the benzisothiazolinone-1-dioxide derivative of the invention, with the formula:



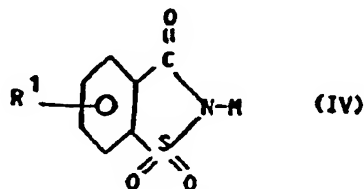
where R¹ is a hydrogen atom or a C₁-C₆ alkyl or alkoxy group,

- R² is an optionally substituted monovalent C₆-C₂₀ alkyl or alkenyl group,

- R² is a group with the formula:



all as defined before by reacting an alkali metal derivative of benzisothiazolinone-1-dioxide having the formula:



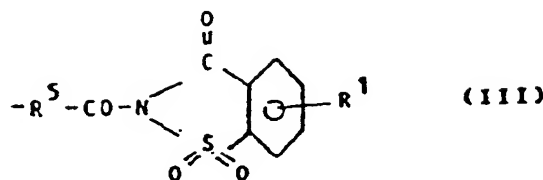
where R¹ is as defined before, and M is an alkali metal with an acid chloride having the formula:



wherein R² is the same as before.

The benzisothiazolinone-1-dioxide derivative with formula I in which R¹ is as defined above and R² repre-

sents the group with formula (III)



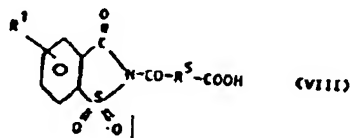
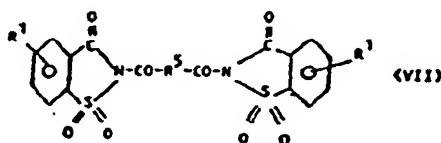
where R^1 and R^5 are as defined before can be prepared by reacting an alkali metal derivative of a benzisothiazolinone-1-dioxide having formula (IV) with an acid chloride represented by:



where R^5 is as defined before, the alkali metal being preferably sodium, but potassium can also be used.

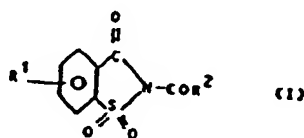
The reaction between the alkali metal derivative and the acid chloride can be carried out in both cases by refluxing the alkali metal derivative of the benzisothiazolinone-1-dioxide (IV) with the acid chloride (V) or (VI) in a suitable solvent such as tetrahydrofuran, with stirring. The product formed can then be isolated by filtration and purified by recrystallization from a suitable solvent such as ethanol.

When an acid chloride with formula (VI) is used, the reaction leads to two different products (VII) and (VIII):



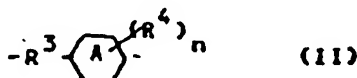
which can be separated from each other by high-pressure liquid chromatography.

The present invention also relates to the new benzisothiazolinone-1-dioxide derivatives themselves with the formula:



where R^1 is a hydrogen atom or a C_1 - C_5 alkyl or alkoxy group, and

- R^2 is a monovalent C_8 - C_{20} alkyl or C_8 - C_{20} alkenyl group optionally substituted with at least one OH or COOH group, with the proviso that if R^2 is unsubstituted alkyl it is C_{11} - C_{20} alkyl, or
- R^2 is a group with the formula:



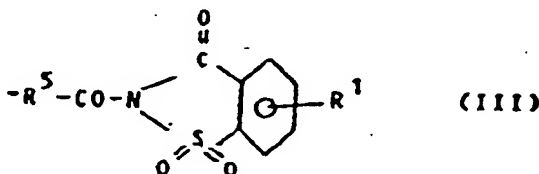


where R³ is a divalent straight or branched C₂-C₈ aliphatic group

R⁴ is OH, a C₁-C₄ alkyl group or a C₁-C₄ alkoxy group

n is zero or an integer in the range of 1-5, and when n > 1, the R⁴ groups can be different, or else:

- R² is a group with the formula:



where R¹ is as defined before and R⁵ is a divalent straight or branched C₈-C₂₀ aliphatic group.

This invention also embraces methods of treatment comprising administration of compositions of this invention as specified above, especially cosmetic treatment by topical application.

The pharmaceutical compositions of this invention may be solutions, suspensions, emulsions, ointments, creams, powders, lotions or gels, with non-toxic carriers or vehicles and possibly also additives and excipients.

The compounds of the invention can thus be incorporated in conventional excipients such as polyethylene glycols, waxes, fats, stearic substances, talc, alcohols, vegetable oils (e.g. sweet or expressed almond oil), mineral oils, wetting agents, thickeners, preservatives, perfumes and colorants.

These pharmaceutical compositions are intended for oral, parenteral, and - most often - local or topical administration.

These compositions can be used to treat or prevent any undesirable biological or pathological change caused by elastase, such as:

- the degradation of the cutaneous elastic fibres due to ageing or to exposure to the sun
- lysis of the pulmonary elastic fibres due to smoking, ageing and various disorders
- emphysema
- the progressive lysis of the elastic layers in the arterial walls during the development of arterio-sclerosis
- arterial disorders due to ageing
- inflammatory foci
- destruction of tissues (e.g. ulcers and necrosis)
- periodontal disorders (degeneration of the gum)
- certain disorders of the bones and joints
- the growth of tumours and the formation of metastases.

As mentioned before, the compounds of the invention can also be used as cosmetics intended to counteract the undesirable effects of elastase on the skin, such as ageing. These cosmetics are essentially intended for application to the skin and can be e.g. solutions, emulsions, creams, ointments, powders, lotions, gels, soaps, milks, face packs, aerosols or bath oils. In the case of emulsions, it is best to use the water-in-oil type, containing the compound of the invention solubilized in the oil phase. These compositions can be prepared by the conventional methods, using the carriers, excipients and additives normally incorporated in such compositions.

The concentration of the new derivative (I) in the composition is chosen according to its activity and the effect required. When intended for local administration once or twice a day, the composition can contain the compound of the invention in a concentration of 0.1 to 5 wt-%.

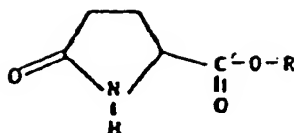
The cosmetic and pharmaceutical compositions according to the invention that are intended for local application may also contain penetration enhancers or penetration potentiators, which can raise the beneficial effect of the elastase inhibitor by improving its diffusion through the epidermis until it reaches its site of action in the stratum corneum.

These penetration enhancers can act in different ways. For example, they can improve the distribution of the elastase inhibitor on the surface of the skin. Alternatively, they can improve its distribution in the skin after local application, thus promoting the migration of the elastase inhibitor within the stratum corneum. The penetration enhancers may also raise the efficiency of the elastase inhibitor by other mechanisms.

Consequently, the pharmaceutical and cosmetic compositions according to the invention can optionally comprise up to 30 wt-% and preferably 0.1-25 wt-% of a penetration enhancer, examples of which are listed below.

- 2-methylpropanol-2
- 2-propanol
- ethyl 2-hydroxypropanoate
- ethyl polyoxyethylene hexane-2,5-diol ether
- 5 – di-(2-hydroxypropyl) ether
- pentane-2,4-diol
- acetone
- methyl polyoxyethylene ether
- 2-hydroxypropionic acid
- 10 – 2-hydroxyoctanoic acid
- 1-propanol
- 1,4-dioxan
- tetrahydrofuran
- 1,4-butanediol
- 15 – propylene glycol dipelargonate
- polyoxypropylene 15-stearyl ether
- octanol
- polyoxyethylene ester of oleyl alcohol
- dioctyl adipate
- 20 – dicapryl adipate
- diisopropyl adipate
- diisopropyl sebacate
- dibutyl sebacate
- diethyl sebacate
- 25 – dimethyl sebacate
- dioctyl sebacate
- dibutyl suberate
- dioctyl azeleate
- dibenzyl sebacate
- 30 – dibutyl phthalate
- dibutyl azeleate
- ethyl myristate
- dimethyl azeleate
- butyl myristate
- 35 – urea
- diethyl-m-toluamide
- 1-dodecylazacycloheptan-2-one
- dibutyl succinate
- dodecyl phthalate
- 40 – decyl oleate
- ethyl caproate
- ethyl salicylate
- isopropyl palmitate
- ethyl laurate
- 45 – 2-ethylhexyl pelargonate
- isopropyl isostearate
- butyl laurate
- benzyl benzoate
- butyl benzoate
- 50 – hexyl laurate
- ethyl caprate
- ethyl caprylate
- butyl stearate
- benzyl salicylate
- 55 – 2-hydroxypropanoic acid
- 2-hydroxyoctanoic acid.

Other substances that promote the penetration of the active ingredient into the skin include the esters of pyrogallonic acid having the formula:

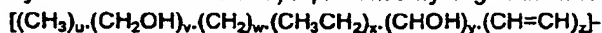


(IX)

where R is either a C₁-C₃₀ alkyl group or it is the group:



where T' and T'' (which may be identical or different) represent a hydrogen atom or the following group:



where u is zero or 1

v is zero, 1 or 2

w is zero or an integer in the range of 1-21

x is zero or an integer in the range of 1-4

y is zero, 1 or 2

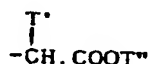
z is zero or an integer in the range of 1-22

and u+v+w+x+y+z is an integer in the range of 1-22, but when the CH=CH group is present, the total number of carbon atoms in the above group is 10-22.

The following compounds are suitable examples of pyroglutamic acid esters in which the R group, featuring in formula (IX), is a C₁-C₃₀ alkyl group:

- methyl pyroglutamate
- ethyl pyroglutamate
- n-propyl pyroglutamate
- n-butyl pyroglutamate
- n-heptyl pyroglutamate
- n-octyl pyroglutamate
- n-nonyl pyroglutamate
- n-decyl pyroglutamate
- n-undecyl pyroglutamate
- n-dodecyl pyroglutamate
- n-tridecyl pyroglutamate
- n-tetradecyl pyroglutamate
- n-hexadecyl pyroglutamate
- n-octadecyl pyroglutamate
- n-eicosyl pyroglutamate
- isopropyl pyroglutamate
- 2-methylhexyl pyroglutamate
- 2-ethylhexyl pyroglutamate
- 3,7-dimethyloctyl pyroglutamate
- 2-hexyldodecyl pyroglutamate
- 2-octyldodecyl pyroglutamate
- 2,4,4-trimethyl-1-pentyl pyroglutamate and
- methyloctyl pyroglutamate.

The preferred esters are those in which R is a straight-chain or branched alkyl group with 1-14 and preferably 1-6 carbon atoms. Other preferred examples of pyroglutamic acid esters are those in which R represents the group:



where the symbols T' and T'' stand for the group:



and which comprise saturated or unsaturated straight-chain or branched C₁-C₂₂ aliphatic groups such as the

alkyl groups: methyl, ethyl, propyl, isopropyl, butyl, isobutyl, n-valeryl, isovaleryl, n-caproyl, n-heptyl, n-caprylyl, n-capryl, lauryl, myristyl, palmityl, stearyl and arachidyl group, and the C₁₀-C₂₂ alkenyl groups: linoleyl, linolenyl, γ-linolenyl, arachidonyl and columbinyl group.

Other examples of these groups comprise hydroxyalkyl radicals with 1-22 carbon atoms, such as the hydroxymethyl, 2-hydroxyethyl, 2-hydroxy-n-propyl, 3-hydroxy-n-propyl, 2-hydroxy-n-butyl, 3-hydroxy-n-butyl, 4-hydroxy-n-butyl, 5-hydroxy-n-valeryl, 6-hydroxy-n-caproyl, 2,3-dihydroxy-n-propyl, 2,3-dihydroxy-n-butyl and 12-hydroxystearyl group.

This list is not exhaustive, and other alkyl or substituted alkyl groups can be added to it as further examples of T' and T''.

Other specific examples of pyroglutamic acid esters that are particularly suitable for use as penetration enhancers are as follows:

- 2-(pyroglutamoyloxy)-propionic acid
- methyl 2-(pyroglutamoyloxy)-acetate
- ethyl 2-(pyroglutamoyloxy)-n-propionate
- ethyl 2-(pyroglutamoyloxy)-n-butyrate
- ethyl 2-(pyroglutamoyloxy)-isobutyrate
- ethyl 2-(pyroglutamoyloxy)-n-valerate
- ethyl 2-(pyroglutamoyloxy)-n-caproate
- ethyl 2-(pyroglutamoyloxy)-n-heptylate
- ethyl 2-(pyroglutamoyloxy)-n-caprylate
- ethyl 2-(pyroglutamoyloxy)-n-pelargonate
- ethyl 2-(pyroglutamoyloxy)-3-hydroxybutyrate
- isopropyl 2-(pyroglutamoyloxy)-n-propionate
- isopropyl 2-(pyroglutamoyloxy)-n-caprylate
- n-propyl 2-(pyroglutamoyloxy)-n-propionate
- n-propyl 2-(pyroglutamoyloxy)-n-caprylate
- stearyl 2-(pyroglutamoyloxy)-n-propionate
- 12-hydroxystearyl 2-(pyroglutamoyloxy)-n-propionate
- stearyl 2-(pyroglutamoyloxy)-n-stearate
- palmityl 2-(pyroglutamoyloxy)-n-propionate
- linoleyl 2-(pyroglutamoyloxy)-n-propionate
- linoleyl 2-(pyroglutamoyloxy)-n-caprylate
- lauryl 2-(pyroglutamoyloxy)-n-caprylate
- stearyl 2-(pyroglutamoyloxy)-n-caprylate
- glyceryl mono-2-(pyroglutamoyloxy)-n-propionate
- glyceryl mono-2-(pyroglutamoyloxy)-n-caprylate, and
- glyceryl di-2-(pyroglutamoyloxy)-n-propionate.

These lists of specific examples of pyroglutamic acid esters are not exhaustive, and many other examples with the overall structure of these esters could be mentioned.

Other examples of penetration enhancers are as follows:

- dimethylsulphoxide
- N,N-dimethylacetamide
- N,N-dimethylformamide
- 2-pyrrolidone
- 1-methyl-2-pyrrolidone
- 5-methyl-2-pyrrolidone
- 1,5-dimethyl-2-pyrrolidone
- 1-ethyl-2-pyrrolidone
- phosphine oxides
- sugar esters, and
- tetrahydrofurfuryl alcohol.

Other features and advantages of the present invention will emerge from the following non-limitative examples, given here to illustrate the present invention.

Example 1 - Preparation of 2-lauroylbenzothiazolinone-1-dioxide

21.8 g (0.1 mole) of lauroyl chloride and 22.66 g (0.11 mole) of dry sodium benzothiazolinone-1-dioxide were refluxed for 4 h in 40 ml of tetrahydrofuran, with mechanical stirring. The reaction mixture was filtered,

the filtrate was concentrated under vacuum, and the product was recrystallized from ethanol and dried in air. This gave 30 g of 2-lauroyl-benzisothiazolinone-1-dioxide (m.p. 85°C).

Examples 2-6

The method described in Example 1 was used to prepare various compounds with formula I, in which the groups denoted by R¹ and R² are shown in Table 1. The acid chloride used here was myristoyl chloride in Example 2, palmitoyl chloride in Example 3, stearoyl chloride in Example 4, decanoyl chloride in Example 5 and undecenoyl chloride in Example 6. The melting points of the compounds obtained are given in Table 1.

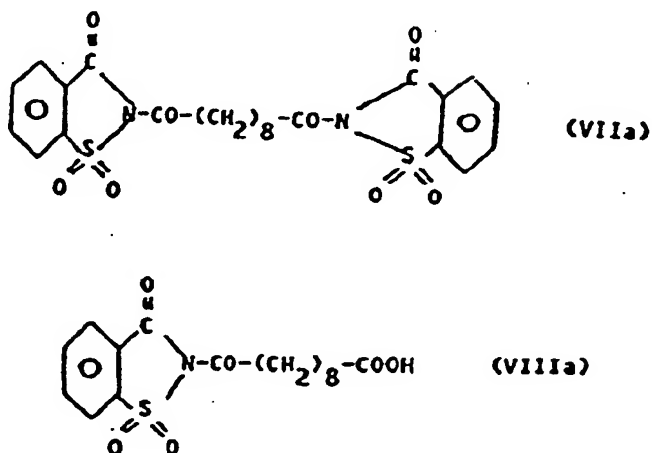
Example 7 - Preparation of 2-[(3,4)-dimethoxycinnamoyl]-benzisothiazolinone-1-dioxide

227 g (0.1 mole) of 1,4-dimethoxycinnamoyl chloride and 22.66 g (0.11 mole) of anhydrous sodium benzisothiazolinone-1-dioxide were refluxed for 5 h in 150 ml of dry tetrahydrofuran, with mechanical stirring. The reaction mixture was then filtered, the filtrate was concentrated under vacuum, and the product obtained was recrystallized from ethanol, giving 22 g of 2-[(3,4)-dimethoxycinnamoyl]-benzisothiazolinone-1-dioxide (m.p. 171°C).

Examples 8-10

The method described in Example 7 was used to prepare various compounds with formula I, in which the groups denoted by R¹ and R² are shown in Table 1. The acid chloride used here was cinnamoyl chloride in Example 8, dihydrocinnamoyl chloride in Example 9, and p-methoxyphenylbutyryl chloride in Example 10. The melting points of the compounds obtained are given in Table 1.

Example 11 - Preparation of compounds (VIIa) and (VIIIa): 1,10-decanedioyl-bis-(2-benzisothiazolin-3-oxo-1,1-dioxide and 1-carboxynonanoyl-10-(2-benzisothiazolin-3-oxo-1,1-dioxide)



A mixture of 9 g (0.05 mole) of sebacoyl chloride and 20.6 g (0.1 mole) of sodium benzisothiazolinone-1-dioxide was refluxed for 4 h. The reaction mixture was then filtered, and the filtrate was concentrated under vacuum. The resulting compounds VIIa and VIIIa were separated by preparative high-pressure liquid chromatography, using a reversed-phase C18 column and a water/acetonitrile gradient. The R_f value of compounds (VIIa) and (VIIIa) was respectively 0.8 and 0.2 when eluted on silica with a 99:1 chloroform-methanol mixture (by volume).

Both the mixture of compounds VIIa and VIIIa and each of them separately can be used as a elastase inhibitor.

Example 12 - Inhibition of human leucocytic elastase

The inhibition test was carried out by using succinyl-trialanine p-nitroanilide as a synthetic substrate. Human leucocytic elastase, used at a concentration of 1 µg/ml, was first pre-incubated for 15 min with the compounds of the invention, used at a concentration of 0.5, 5, 10, 25 and 50 µg/ml. These compounds were added in the form of a solution in acetone, the final acetone concentration of the reaction mixture being 1%.

The degree of hydrolysis of the substrate was determined by measuring the amount of p-nitroaniline released, using a Philips PO 8700 spectrophotometer at 410 nm. The 50% inhibitory concentration (IC_{50}) in mole/litre was then determined by a graphical method, using the inhibition values obtained with the different concentrations of the substrate and the inhibitor. Table 1 shows the IC_{50} values obtained for the compounds of the invention prepared in Examples 1-5. It can thus be seen that the inhibitory action of these compounds increases with the number of carbon atoms in the R^2 group on going from C_9 to C_{17} .

Table 1

Example No.	R^1	R^2	M. p., °C	IC_{50} , mole/l
1	H	$C_{11}H_{23}$	85	1.1×10^{-5}
2	H	$C_{13}H_{27}$	89	9.2×10^{-6}
3	H	$C_{15}H_{31}$	94	2.1×10^{-6}
4	H	$C_{17}H_{35}$	90	4.7×10^{-6}
5	H	C_9H_{19}	84	8.6×10^{-5}
6	H	$C_{10}H_{19}$	75	7.5×10^{-5}
7	H	$-CH=CH-\text{C}_6\text{H}_4-\text{OCH}_3$	171	5×10^{-6}
8	H	$-CH=CH-\text{C}_6\text{H}_5$	222	9×10^{-5}
9	H	$-CH_2-CH_2-\text{C}_6\text{H}_5$	142	4.8×10^{-5}
10	H	$-(CH_2)_3-\text{C}_6\text{H}_4-\text{OCH}_3$	142	3.6×10^{-5}

Example 13 - Protective action of 2-lauroylbenzothiazolinone-1-dioxide on elastin and its inhibition of human leucocytic elastase

Three series of tests were carried out here to illustrate the manner by which the compound prepared in Example 1 inhibits the elastase.

1) **Inhibitory action.** The elastase, used in a concentration of 1 µg/ml, was incubated for 10 min with different amounts of 2-lauroylbenzothiazolinone-1-dioxide in a 100-mM tris-HCl buffer (pH 8.4) containing 0.01%

of Brij 35 and 0.01% of NaN_3 . Tritiated insoluble elastin extracted from the nuchal ligament was then added in a concentration of 75 $\mu\text{g/ml}$, corresponding to a radioactivity level of 2.2×10^8 counts per minute (cpm) per ml.

2) **Protective action.** The substances and concentrations were the same as above, but the insoluble elastin was incubated with 2-lauroylbenzothiazolinone-1-dioxide for 30 min before the introduction of human leucocytic elastase.

3) **Third series.** Again the same substances and concentrations were used as before, but the insoluble elastin was first incubated with 2-lauroylbenzothiazolinone-1-dioxide for 10 min. The mixture was then centrifuged, the supernatant was discarded, and the residue was suspended in the buffer that contained human leucocytic elastase.

The degree of the hydrolysis of elastin was calculated in all three cases after incubation with elastase for 7 h at 37°C by determining the radioactivity in the solubilized peptides derived from the elastin.

The aim of the first series was to determine the direct ability of 2-lauroylbenzothiazolinone-1-dioxide to inhibit the activity of human leucocytic elastase. The results show that, when used in a concentration of 45 $\mu\text{g/ml}$, 2-lauroylbenzothiazolinone-1-dioxide inhibits the elastin-cleaving activity of human leucocytic elastase by 80%, the value of IC_{50} being 7.5×10^{-5} M.

The second and third series of investigations showed that the compound of the invention also protects the insoluble elastic fibres from the action of human leucocytic elastase with a maximum inhibition of 50% when used in a concentration of 50-100 $\mu\text{g/ml}$, the IC_{50} value being $1.3\text{-}2.9 \times 10^{-4}$ M.

Example 14 - Protective action of 2-lauroylbenzothiazolinone-1-dioxide on the elastin in rabbit skin

Frozen biopsy specimens of rabbit skin having a thickness of 6 μ were treated either with human leucocytic elastase in a concentration of 15 $\mu\text{g/ml}$ or with a mixture of this and 2-lauroylbenzothiazolinone-1-dioxide, prepared in Example 1 and used in a concentration of 350 $\mu\text{g/ml}$.

The segments of rabbit skin were incubated for 1.5 h at 37°C . In the control experiment, they were incubated only with the buffer (100-mM tris-HCl, 0.1% of Brij 35, pH 8) under the same conditions.

Thin sections of the skin specimens were then fixed for 2 min in 95% ethanol and stained for 3 h by a modified Verhoeff method, described by Godeau et al. [cf. *Pathol. Biol.*, **32** (1944) pp. 215-6]. After a suitable contrast treatment, the surface density of the elastic fibres was determined by automatic image analysis, carried out directly on the microscope slides.

In the absence of elastase, the volume fraction V occupied by the cutaneous elastic fibres was $6.25 \pm 0.5\%$. After treatment with human leucocytic elastase, this value was only $4.1 \pm 0.8\%$. When the elastase was first incubated with 2-lauroylbenzothiazolinone-1-dioxide, however, the value of V was $6.00 \pm 1\%$, which indicates a virtually complete (96%) protection from the action of elastase.

Example 15 - Determination of the inhibition of other serine-containing proteases

The inhibition exerted by the following two compounds on serine-containing proteases other than human leucocytic elastase was determined in this Example:

- 2-butyryl-benzothiazolinone-1-dioxide (B) and
- 2-palmitoyl-2-benzothiazolinone-1-dioxide (P).

More specifically, the activity of a) pig pancreatic elastase, b) trypsin, c) thrombin and d) plasmin was determined in the presence of these inhibitors and of the synthetic substrates: a) succinyltrialanine p-nitroanilide, b) N-benzoyl-DL-arginine p-nitroanilide, c) N-p-tosyl-gly-pro-arg p-nitroanilide and d) N-p-tosyl-gly-pro-lys p-nitroanilide, respectively.

Each enzyme was first incubated for 15 min with 0-50 $\mu\text{g/ml}$ of compound B or P mentioned above, which had been dissolved in acetone as in the test with human leucocytic elastase, the final concentration of this solvent in the reaction mixture being 1%.

The appropriate substrate was then added to each enzyme, and its hydrolysis was monitored with a Philips 8700 spectro-photometer at 410 nm by measuring the amount of p-nitroaniline appearing in the medium.

The results were used to plot the inhibition of the enzymatic activity against the amount of inhibitor present in the medium. These curves were then used to find the median inhibitory concentration (IC_{50}) in mole/l, which inhibits 50% of the enzymatic activity.

Inhibition of human leucocytic elastase was also determined, as in Example 12.

To be able to compare the inhibitory power of the compounds tested on the various enzymes used, the E/IC_{50} value was calculated, where E is the concentration of the enzyme in the reaction mixture (also expressed in mole/l). The higher the value of this quotient, the stronger the inhibitory activity, and therefore the lower the number of moles of the inhibitor that are needed to obtain a 50% inhibition for the same number of moles of

the enzyme.

The results listed in Table 2 show that palmitoylbenzisothiazolinone-1-dioxide is a better inhibitor than butyrylbenzisothiazolinone-1-dioxide, irrespective of the enzyme used. Furthermore, these inhibitors show different specificities for the different serine proteases employed. Thus, 2-butyrylbenzisothiazolinone-1-dioxide inhibits human leucocytic elastase and pig pancreatic elastase more strongly than it inhibits thrombin; it has no effect on plasmin and in fact activates trypsin. 2-Palmitoyl-2-benzisothiazolinone-1-dioxide inhibits human leucocytic elastase, pig pancreatic elastase and trypsin 20-40 times as strongly as it inhibits thrombin. This indicates that the compound according to the invention, i.e. 2-palmitoyl-benzisothiazolinone-1-dioxide, is much more efficient than 2-butyrylbenzisothiazolinone-1-dioxide, in which the hydrocarbon chain contains fewer than nine carbon atoms.

Table 2

	E/IC ₅₀	
	Butyrylbenziso- thiazolinone- 1-dioxide	Palmitoylbenziso- thiazolinone- 1-dioxide
Human leucocytic elastase	7×10^{-4}	8.1×10^{-9}
Pig pancreatic elastase	3.7×10^{-6}	3.2×10^{-9}
Trypsin	activator	4.2×10^{-9}
Thrombin	2.3×10^{-6}	1.8×10^{-4}
Plasmin	inactive	

Examples 16-21 illustrate some cosmetic compositions containing elastase inhibitors according to the present invention.

Example 16

This Example illustrates a gel for the treatment of hair, this product containing the compound mentioned in Example 2, i.e. 2-myristoylbenzisothiazolinone-1-dioxide, and having the following composition.

	Amount, wt-%
Emulsifier	20.00
Silicone oil	20.00
Inhibitor from Example 2	2.00
Sodium hydroxide	4.55
1,3-Butanediol	11.00
Lactic acid	5.00
Water	<u>37.45</u>
	100.00

Example 17

This Example illustrates a face pack containing the inhibitor used in Example 4, i.e. 2-stearoylbenzothiazolinone-1-dioxide, and having the following composition, the product being prepared by mixing the ingredients together.

		<u>Amount, %</u>
10	Kaolin	35.00
	Bentonite	5.00
	Cetyl alcohol	2.00
	Potassium dodecyl sulphate	1.00
15	Glycerol	10.00
	Nipagin M	0.10
	Inhibitor from Example 4	5.00
20	Perfume	5.00
	Water	<u>36.90</u>
		100.00

Example 18

This Example illustrates a lotion suitable for the treatment of nails, containing the inhibitor used in Example 6, i.e. 2-undecenylbenzothiazolinone-1-dioxide, and having the following composition, the lotion (which had a pH of 4.4) being prepared by homogenizing the mixture of its ingredients.

		<u>Amount, %</u>
	Inhibitor from Example 6	6.00
35	Sodium hydroxide	1.50
	Ethanol	10.00
	1,2-Propanediol	55.00
40	Water	<u>27.50</u>
		100.00

Example 19

This Example illustrates a skin cream formed by a water-in-oil emulsion and containing the inhibitor from Example 1, i.e. 2-lauroylbenzothiazolinone-1-dioxide in its continuous oil phase, the composition of the cream being as follows.

		<u>Amount, wt-%</u>
	Silicones	24.00
5	Sodium chloride	2.00
	Inhibitor from Example 1	3.00
	Lactic acid	5.00
10	Humectants	5.00
	Bleaching agent	0.15
	Preservatives	0.05
	Oil of evening primrose	3.00
15	Sunscreens	4.00
	Bactericides	0.30
	Water	<u>53.50</u>
20		100.00

This skin cream, which had a pH of 4, was prepared by mixing the silicones, the bleaching agent and the preservatives together, adding a mixture of the other ingredients in small portions, and homogenizing the product.

25 Example 20

This Example illustrates a water-in-oil type cream, which contained sunscreens in its continuous oil phase, together with the inhibitor from Example 7, i.e. 2-(3,4-dimethoxy-cinnamoyl)-benzisothiazolinone-1-dioxide.
30 This cream had the following composition.

		<u>Amount, %</u>
	Silicones	24.00
35	Humectants	10.00
	Bleaching agent	0.15
	Preservatives	0.05
40	Oil of evening primrose	3.00
	Sunscreens	4.00
	Bactericides	0.30
	Inhibitor from Example 7	1.00
45	Ammonium hydroxide	2.00
	Ammonium chloride	2.00
	Lactic acid	5.00
50	Water	<u>48.50</u>
		100.00

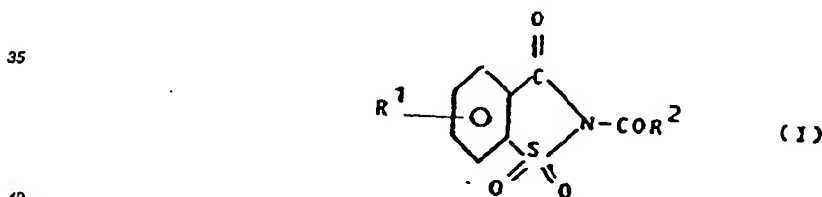
55 Example 21

This Example illustrates a water-in-oil type cream that contained sunscreens in its continuous oil phase, together with the inhibitor with formula (VIIa) from Example 11, i.e. 1, 10-decanedioyl-bis-(benzisothiazolin-3-oxo-1,1-dioxide), the cream having the following composition.

	Amount, %
Silicones	24.00
Humectants	10.00
5 Bleaching agent	0.15
Preservatives	0.05
Oil of evening primrose	3.00
10	
Sunscreens	4.00
Bactericides	0.30
15 Inhibitor from Example	
II (with formula VIIa)	1.00
Ammonium hydroxide	2.00
20 Ammonium chloride	2.00
Lactic acid	5.00
Water	<u>48.50</u>
	100.00
25	

Claims

- 30 1. A composition comprising (i) an elastase inhibitor which is at least one benzisothiazolinone-1-dioxide derivative having the formula:



where R¹ is a hydrogen atom or a C₁-C₆ alkyl or alkoxy group, and

- R² is a monovalent C₆-C₂₀ alkyl or C₆-C₂₀ alkenyl group, optionally substituted with OH or COOH group,

45 - R² is a group with the formula:



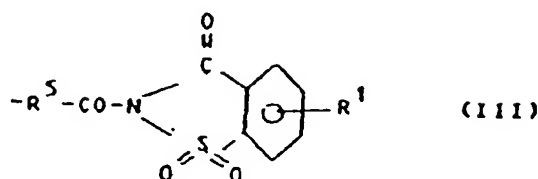
where R³ is a divalent straight or branched C₂-C₈ saturated or ethylenically unsaturated aliphatic

group  is an aromatic nucleus

R⁴ is OH, a C₁-C₄ alkyl or a C₁-C₄ alkoxy group

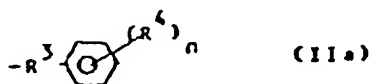
n is zero or an integer in the range of 1-5, and when n > 1, the R⁴ groups can be different,

or else
 - R² is a group with the formula:



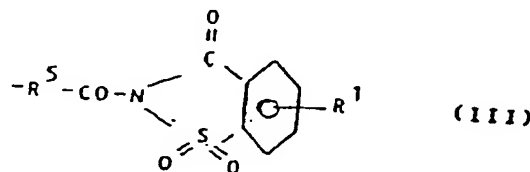
where R¹ is the same as above, and
 R⁵ is a divalent straight or branched C₈-C₂₀ saturated or ethylenically unsaturated aliphatic group,
 and (ii) a pharmaceutically or cosmetically acceptable carrier or vehicle.

- 15
2. A composition as claimed in claim 1, wherein R² is a C₁₁-C₂₀ alkyl group.
 3. Composition as claimed in claim 1 or claim 2, wherein R¹ is a hydrogen atom, and R² is an alkyl group substituted with COOH, or an alkenyl group.
 - 20
 4. Composition as claimed in claim 3, wherein R¹ is a hydrogen atom, and R² is chosen from nonyl, undecyl, tridecyl, pentadecyl, heptadecyl, carboxyoctyl and dec-9-enyl groups.
 5. Composition as claimed in claim 1 or claim 2, wherein R¹ is a hydrogen atom, and R² is a group with the formula:
- 25



where R³ is a saturated or olefinically unsaturated divalent C₂-C₆-aliphatic group, R⁴ is a hydroxyl group or a C₁-C₄ alkoxy group, and n = 1 or 2.

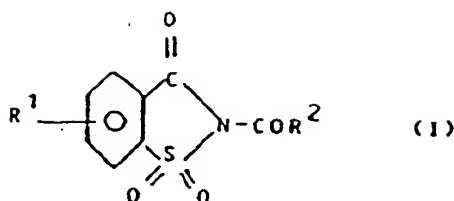
- 35
6. Composition as claimed in claim 7, wherein R² is a 3,4-dimethoxycinnamoyl, cinnamoyl, dihydrocinnamoyl or p-methoxyphenylbutyryl group.
 7. Composition as claimed in claim 1 or claim 2, wherein R¹ is a hydrogen atom, and R² is a group with the formula:
- 40



where R¹ is hydrogen and R⁵ is a divalent saturated aliphatic group.

- 50
8. Composition as claimed in any one of the preceding claims which contains 0.1 to 5% by weight of compound of formula (I).
 9. A benzisothiazolinone-1-dioxide derivative with the formula:
- 55

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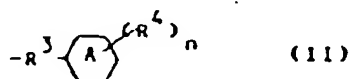


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where R¹ is a hydrogen atom or a C₁-C₆ alkyl or alkoxy group, and

- R² is a monovalent C₈-C₂₀ alkyl or C₉-C₂₀ alkenyl group optionally substituted with at least one OH or COOH group, with the proviso that if R² is unsubstituted alkyl it is C₁₁-C₂₀ alkyl, or
- R² is a group with the formula:

15



20

(where R³ is a divalent straight or branched C₂-C₆ aliphatic group

A is an aromatic nucleus

R⁴ is OH, a C₁-C₄ alkyl or a C₁-C₄ alkoxy group

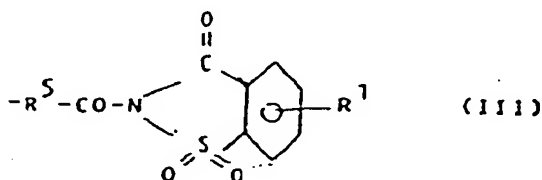
n is zero or an integer in the range of 1-5, and when n > 1, the R⁴ groups can be dif-

ferent) or else

25

- R² is a group with the formula:

30



35

where R¹ is the same as above, and

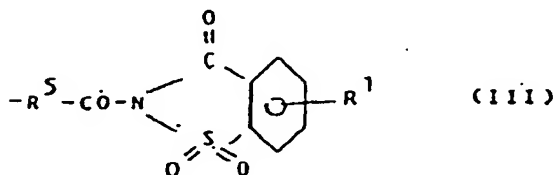
R⁵ is a divalent straight or branched C₈-C₂₀ aliphatic group.

40

10. A benzisothiazolinone-1-dioxide derivative as claimed in claim 12, wherein R² is a 3,4-dimethoxycinnamoyl, cinnamoyl, dihydrocinnamoyl or p-methoxyphenylbutyryl group.

11. A benzisothiazolinone-1-dioxide derivative as claimed in claim 12, wherein R² is a group having the formula:

45



50

where R¹ is hydrogen and R⁵ is a -(CH₂)₈- group.

55

12. A method of cosmetic treatment of the human skin comprising applying topically thereto a composition according to any one of claims 1 to 11.

13. Use of a benzisothiazolinone-1-dioxide derivative as defined in any one of claims 1 to 7 for the manufacture of a medicament or a cosmetic composition.

European Patent
Office

EUROPEAN SEARCH REPORT

Application Number

EP 91 30 1895

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 5)
D, X	US-A-4 195 023 (D. MULVEY) * Whole document *	1	C 07 D 275/06 A 61 K 7/48 A 01 K 31/425
A	---	9	
A	US-A-4 276 298 (H. JONES) * Whole document *	1	
A	JOURNAL OF THE CHEMICAL SOCIETY, 1957, pages 492-493, London, GB; E. STEPHEN et al.: "The preparation of 2-acyl-3-oxo-4 : 5-benzo-1 : 2-thiazole 1 : 1 - dioxides (N-acylsaccharins) for the identification of monocarboxylic acids" * Page 493 *	9	
The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (Int. Cl. 5)
			C 07 D 275/00 A 61 K 7/00
Place of search THE HAGUE		Date of completion of the search 17-05-1991	Examiner HENRY J.C.
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure F : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons A : member of the same patent family, corresponding document			

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